Litter species richness and composition effects on fungal richness and community structure in decomposing foliar and root litter

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13 **Abstract**

14 Litter species richness influences fungal diversity because of resource heterogeneity. Litter

15 species composition effects on decomposition have been studied mostly in aboveground litter.

16 However, little is known about the effects of litter diversity of roots and litter type effect on

17 fungal diversity in general. We addressed litter species composition and richness effects on

18 diversity of ectomycorrhizal (EcM), saprotrophic and pathogenic fungi in decomposing foliar

19 and root litter in the Satakunta forest diversity experiment by varying litter species richness

20 from one to four species in 560 litter bags incubated under tree canopies. After one year of

21 decomposition, there were no significant non-additive effects of litter mixtures on mass loss.

22 Litter species composition was the strongest predictor for saprotroph and plant pathogen

23 community structure in foliar litter, but its effect on root litter fungal composition was much

24 weaker. Litter diversity strongly enhanced fungal richness in foliar but not in root substrate.

25 We found no evidence for host litter preferences in host-specific EcM fungi. Our study

26 suggests that litter species richness and especially certain litter species may influence

27 decomposition through modifying fungal community composition both in foliar and root

28 litter.

29

30 Key words: fungal community, fungal richness, foliar litter, root decomposition, litter

31 mixture, litter mass loss.

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1. Introduction

Functioning of above- and belowground components of terrestrial ecosystems are influenced by feedbacks between plants, root associated organisms and decomposers (Wardle et al., 2004). In mixed forest ecosystems, individual trees affect decomposition directly through species-specific litter quality (McClaugherty et al., 1985; Hobbie, 1992; Silver and Miya, 2001) and indirectly through modified micro-environmental conditions and soil biota composition (Joly et al., 2017). In particular, Hobbie et al. (2006) showed that tree species identity affects decomposition via both litter chemistry and effects on soil temperature. The strength of feedback to plants depends on plant traits and interactions between root- and litter-associated microbes (Cairney and Meharg, 2002; Ke et al., 2015).

Together with environmental variables such as temperature and moisture, plant traits such as leaf nutrient and lignin concentration are strong predictors of litter decomposition in forest ecosystems (Hättenschwiler, 2005; Cornwell et al., 2008; Bani et al., 2018). Due to litter quality effects, broadleaf litter is expected to decompose faster than coniferous litter (Prescott et al., 2000). Plant litter is often decomposed more rapidly in the vicinity of the mother plant due to specialized decomposer community, termed as 'home-field advantage effect' (Ayres et al., 2009; Veen et al., 2015). Home-field advantage has been found to be common in forest ecosystems and may cause an average 8% faster litter mass loss in 'home' than 'away' habitats (Ayres et al., 2009). Home-field advantage in decomposition communities has been found to be related to chemical similarity in litter quality rather than related to associations between specific species (Gholz et al., 2000; Veen et al., 2015). Ke et al. (2015) showed that litter decomposability through soil nitrogen (N) availability may determine the strength of plant-soil feedback in communities dominated by arbuscular mycorrhizal fungi.

In forest ecosystems, foliar litter from different plant species becomes usually mixed because of wind and roughly simultaneous litter fall in the autumn (Staelens et al., 2003; Ťupek et al., 2015). Similarly, root litter is mixed, because roots of plant species commonly intermingle and their turnover is usually synchronous (Fogel, 1983; Brassard, 2010). Most root systems of trees in boreal forest ecosystem have been shown to stop activity after leaf-drop in the fall (Burke and Raynal, 1994). Mixing litter of different plant species may cause shifts in microbial communities that may affect decomposition rates (Chapman et al., 2013). In particular, chemically diverse litter mixtures provide decomposers with a varied diet that has strong effects on microbial communities and functional diversity and hence may increase or decrease decomposition rates with positive or negative feedbacks to plants (Gartner and Cardon, 2004; Hättenschwiler et al., 2005). Decomposition patterns of leaf litter mixtures are usually 'non-additive', i.e. not directly predictable based on the biogeochemical processes in parent litter, whereas 'additive' responses in mixtures are predictable from component species (Gartner and Cardon, 2004). Most studies have revealed that litter species composition has a stronger effect on decomposition rates than litter richness *per se* (Wardle et al., 2006; Hoorens et al., 2010; Cuchietti et al., 2014; Handa et al., 2014; Setiawan et al., 2016).

Litter mixing affects microbial abundance and community structure and tends to result in synergistic or antagonistic effects where decomposition rates are faster or slower, respectively, than expected based on decomposition rates of litter of component species (Gartner and Cardon, 2004). However, linking microbial community changes directly to these non-additive patterns in litter mixtures has been elusive (Chapman et al., 2013). It has been shown that litter species composition determines the direction of non-additive effect in the litter mixture (De Marco et al., 2011). It has been suggested that synergistic effects in litter mixtures are caused by fungi-driven nutrient transfer from higher quality litter to the poor one, and antagonistic effects are due to release of inhibitory compounds (Chapman et al., 1988).

81   Berglund and Ågren (2012) showed that the litter mixture decomposes faster than parent

82   litters alone when the litter of the higher quality mineralizes nitrogen fastest. More diverse

83   and patchy plant litter may promote niche specialists and hence support higher microbial

84   diversity (Kubartová et al., 2009; Chapman and Newman, 2010; Santonja et al., 2017). Litter

85   of different tree species develop distinct microbial communities, however, microbial changes

86   may not translate to predictably altered litter decomposition (Aneja et al., 2006; Chapman and

87   Newman, 2010).

88   Root litter represents an important C source for microbial communities (Rasse et al., 2005)

89   and plays a substantial role in plant-soil feedback (Freschet et al., 2013). Due to lower

90   nutrient concentration and higher lignin content, roots are considered to be more recalcitrant

91   than foliar litter and this might have an effect on their microbial degradability (Berg and

92   McClaugherty, 2014; Jacobs et al., 2018). To date most studies on litter decomposition have

93   focused on the foliar component and little attention has been given to understanding the

94   patterns and drivers of the large source of carbon that is found in roots, partly because of the

95   challenges of studying belowground litter. Little is known about the community composition

96   of microbial decomposers of dead roots. For example, Herzog (2017) and Kohout et al. (2018)

97   studied the composition of dead root associated decomposer communities over a two-year

98   period; Fisk et al. (2011) investigated fungal community development on dead roots in

99   disturbed rhizosphere conditions. Several studies have been conducted to analyze if fungal

100   community composition varies between litters of different diversity (Kubartová et al., 2009;

101   Chapman and Newman, 2010; Chapman et al., 2013). However, the effects of foliar litter

102   diversity and particularly root litter diversity on fungal functional groups in forest ecosystems

103   remain largely unknown.

104   Saprotrophs, endophytes, plant pathogens and EcM fungi are the major fungal guilds

105   inhabiting foliar and root litter (Lindahl et al., 2007; Voříšková and Baldrian, 2013; Kohout et

al., 2018). While saprotrophic fungi are the primary decomposers of C-rich biopolymers (Cooke and Rayner, 1984; Talbot et al., 2013), some plant pathogens (Baker and Bateman, 1978; Osono, 2007) and endophytes (Müller et al., 2001; Korkama-Rajala et al., 2008; Kohout et al., 2018) are also potentially important in the initial stages of decomposition, as they often express saprotrophic activity after leaf senescence. EcM fungi take part in organic matter decomposition through mobilizing organically bound nitrogen (Lindahl and Tunlid, 2015), which may lead to direct competition for resources with saprotrophs and result in an overall decrease in decomposition rate (Fernandez and Kennedy, 2016).

Host preference has been observed in endophytes, plant pathogens, saprotrophs and mycorrhizal fungi (Molina et al., 1992; Zhou and Hyde, 2001; Põlme et al., 2018). Of these four groups, mycorrhizal fungi exhibit a high degree of host specificity which is thought to be a result of their coevolution with plants (Brundrett, 2002). Thus, the diversity of belowground organisms who are intimately associated with plant roots (e.g. EcM fungal taxa) are predicted to create stronger aboveground effects (influence on plant productivity, composition, and diversity) compared with saprotrophs with low specificity (Wardle et al., 2004). Also, community composition of EcM fungi might respond strongly to the aboveground communities through litter chemistry (Conn and Dighton, 2000). Tedersoo et al. (2008) showed that EcM fungal community composition in dead wood responds to tree species of woody litter that probably results from chemical differences in substrate. Given the specialization of many EcM fungal species to certain plant host genera (Kennedy et al., 2015; Molina and Horton, 2015), we predict that the relative proportion of host-specific EcM fungi is greater in their host plant's litter than in other tree species' litter due to their long evolutionary history of being exposed to the host plant's litter.

The aim of this study was to determine the ecological relationships between fungal diversity and plant diversity via feedbacks to foliar and root litter. We hypothesized that mass loss rate

131   increases with increasing litter diversity (H1); litter from a single tree species decomposes

132   more rapidly under the trees where it originated (H2); composition of saprotrophs, plant

133   pathogens and EcM fungi is mainly driven by litter species composition (H3); litter species

134   richness enhances fungal richness (H4); and the relative proportion of host-specific EcM

135   fungi is greater in the litter of their intimate host plant (H5).

136

2. Materials and Methods

2.1. Study site and experiment set up

To study litter diversity effects on fungal community composition and richness, a litter decomposition experiment was set up in the Satakunta forest diversity experimental area (www.sataforestdiversty.org) in Finland (61.714°N, 21.983°E). The forest diversity experiment was established in 1999 to study the effects of tree species richness and composition on ecosystem functioning. Experimental plots in three different areas were planted with monocultures, two-, three- and five-species mixtures of silver birch (*Betula pendula*), black alder (*Alnus glutinosa*), Siberian larch (*Larix sibirica*), Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*). Each 20 m x 20 m plot contains 13 rows with 13 trees per row planted at 1.5 m intervals. In mixed species plots, all tree species were planted in equal proportions. In 2013, one replicate of each treatment per area was thinned with tree density reduced by half. In 2014-2015, the annual precipitation was 567-673 mm and annual temperature was 6.7-6.9 °C (www. ilmatieteenlaitos.fi – Pori meteorological station).

In order to minimize spatial effects, we selected two five-species mixture plots (plots 18 and 22 in area 1) to test the effects of tree species identity, foliar and root litter species composition and richness on microbial diversity and litter mass loss. Our block design included 20 tree individuals per plot with a total of 40 focal trees (blocks). Within each of the two plots, we randomly selected 5 individual trees of each tree species except the non-native larch and placed litter bags (see next paragraph) under these trees. Each tree (block) received eight bags of single-species foliar and root litters, two bags of two-species (the same random combination for both foliar and root litters) and two four-species foliar and root litter mixtures. In each block, we used two extra bags of single-species foliar and root litter to increase the power for 'home' versus 'away' comparisons.

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161 Foliar litter was collected by use of litter traps from neighboring monoculture plots and air-

162 dried at room temperature in September, 2014. Roots of the four tree species were excavated

163 from the edges of the two selected plots in order not to disturb the rest of the plot. We selected

164 an equal proportion of fine (≤2 mm diam.) and coarse roots (2-3 mm diam.). All roots were

165 dried at 65 °C for 48 hours to eliminate living EcM fungi, whereas leaves were dried at room

166 temperature to better mimic natural conditions. Heating may eliminate most fungi (Langley et

167 al., 2006) and affect nutrient form, distribution and availability in plant litter (Gray and

168 Dighton, 2006), which renders foliar and root litter effects incomparable in our study.

169 Litter bags (10 x 10 cm) were prepared of polyester (500 µm mesh size) that allowed root and

170 mycelium ingrowth and migration of meiofauna. 1 g of litter was weighed into each bag, with

171 litter species mixes pooled in equal proportion. Both litter substrates were kept as intact as

172 possible. Litter bags were installed above soil surface by use of nails and wire. Ground

173 vegetation and natural litter were cleared to ensure contact with the soil. Litter bags were

174 placed to one side of the tree (directing to a tree of same species), approximately 0.5 m from

175 the tree trunk and 5 cm distance between the bags.

176 For studying the effect of litter richness of one-, two- and four-species mixtures, 400, 80 and

177 80 litter bags were used, respectively. In October 2014, altogether 560 litter bags were

178 installed including 280 bags of foliar and 280 bags of root litter. Litter bags were harvested

179 after 12 months of decomposition in October 2015. We were unable to retrieve four of the

180 litter bags. The litter bags were carefully freed from the adhering soil particles and air-dried at

181 room temperature for 48 hours. Then, the decomposed litter was weighted in sterile conditions

182 and manually crushed in zip-lock plastic bags. 0.20 g of material from each sample was

183 further powdered in 2-ml Eppendorf tubes using two 3-mm tungsten carbide beads in Mixer

184 Mill MM400 (Retsch GmbH, Haan, Germany) at 30 Hz for 5 min for molecular analysis.

185   Initial C, N and phosphorus (P) concentrations in four foliar and four root litter species

186   samples were determined according to Tedersoo et al. (2012) to compare the differences in

187   litter quality between foliar and root litter, and broadleaf and coniferous litter. Litter species

188   and chemical parameters are given in Supplementary Table S1.

189   *2.2. Molecular analysis*

190   DNA was extracted from litter samples using PowerSoil DNA Isolation Kit (MoBio,

191   Carlsbad, CA, USA) following the manufacturer's protocols. PCR was carried out using a

192   mixture of five forward primers ITS3mix1-5 (CANCGATGAAGAACGYRG) (Tedersoo et

193   al., 2014) in equimolar concentration and a degenerate reverse primer ITS4ngsUni (Tedersoo

194   and Lindahl, 2016). The ITS4ngsUni (CCTCCSCTTANTDATATGC) primer was tagged

195   with one of the 110 identifier barcodes (10–12 bases) (Supplementary Table S2) that were

196   modified from those recommended by Roche (Basel, Switzerland) to differ by >3 bases, to

197   start only with adenosine and to comprise similar proportions of adenosine and thymidine

198   (between 30 and 70%) to equalize their affinities in an adapter ligation step (Tedersoo et al.,

199   2014). The PCR mixture comprised 1 μl DNA, 0.5 μl each of the primers (20 μM), 4 μl 5×

200   HOT FIREPol Blend Master Mix (Solis Biodyne, Tartu, Estonia) and 14 μl double-distilled

201   water. PCR was carried out in two replicates in the following thermocycling conditions: an

202   initial 15 min at 95 °C, followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1

203   min, and a final cycle of 10 min at 72 °C. PCR products (typically 350–400 bp) from replicate

204   samples were pooled and their relative quantity was estimated by running 5 μl DNA on 1%

205   agarose gel for 15 min. DNA samples with no visible bands were re-amplified with 35 cycles

206   and DNA samples with strong bands were re-amplified with only 25 cycles. Both negative

207   (sterile water used for PCR mixture) and positive controls (Australian truffles *Reddellomyces*

208   *donkii* specimen MURU5473 and *Dingleya* sp. specimen MURU5844) were included in PCR

209   and sequencing runs. PCR products were pooled at approximately equimolar ratios as

determined by gel band strength, with the exception of negative controls, from which 5 μl were

added. Samples were combined into six libraries (foliar and root litter were kept separate).

Libraries were purified by FavorPrep™ Gel/PCR Purification Kit (Favorgen-Biotech Corp.,

Austria), following the manufacturer's instructions. DNA from each library was quantified

using Qubit® 2.0 Fluorometer (Invitrogen, Life Technologies, CA, USA) and dsDNA High

Sensitivity assay kit (ThermoFisher Scientific, Waltham, USA). DNA concentrations for each

library ranged from 63 ng μl^{-1} to 231 ng μl^{-1}. Amplicons were subjected to adaptor ligation

and Illumina MiSeq sequencing in the Estonian Genome Center (Tartu, Estonia).

2.3. Bioinformatics analysis

Illumina sequencing provided 14,564,313 raw reads. Raw reads were processed using

PipeCraft 1.0 platform (Anslan et al., 2017). Paired-end reads were merged and quality

trimmed using VSEARCH v1.1.11 (Rognes et al., 2016) (trimming options: maximum

expected error rate = 1). The resulting 9,947,496 sequences were re-assigned to samples based

on the tags using mothur v1.36.1 (Schloss et al., 2009). Chimera filtering of the sequences

was performed using VSEARCH based on UNITE v6 reference database (Abarenkov et al.,

2010b) and *de novo* option. To extract the full-length ITS2 subregion for clustering purposes,

reads were processed with ITSx 1.0.9 (Bengtsson-Palme et al., 2013) to remove flanking gene

fragments. Full-length ITS2 reads were assigned to operational taxonomic units (OTUs) by

clustering at 97% similarity threshold with CD-Hit v4.6 (Fu et al., 2012). All OTUs

represented by a single sequence (singletons) were removed. The most abundant sequence of

each cluster was selected as a representative for BLASTn sequence similarity search (word

size=7; gap open=1; gap extension=2; revard=1; penalty=-1) against both INSDc

(International Nucleotide Sequence Databases Collaboration) and UNITE. Also, BLASTn

searches were run against reference sequences of fungi in 99.0% similarity species hypothesis

234　(SH) that include third-party taxonomic and metadata updates (Kõljalg et al., 2013; Nilsson et

235　al., 2014) as implemented in the PlutoF workbench (Abarenkov et al., 2010a).

236　Further filtering of the dataset was performed manually. We used BLASTn output (similarity,

237　e-value and match length/sequence length ratio) for taxonomic assignment as well as positive

238　and negative controls to remove low quality sequences, tag switching errors and

239　contaminants. We considered 10 best matching references for each OTU to annotate taxa as

240　accurately as possible and ran manual BLASTn searches against the INSDC if no reliable

241　taxonomy was revealed. BLASTn e-values $<e^{-50}$ were considered reliable to assign OTUs to

242　kingdoms, whereas OTUs with e-values $>e^{-20}$ were treated as 'unknown' taxa. E-values

243　between e^{-20} and e^{-50} were manually checked against the 10 best matches for accurate

244　assignment. We relied on 98%, 90%, 85%, 80%, and 75% sequence identity as a criterion for

245　assigning OTUs to species, genus, family, order or class level, respectively (Tedersoo et al.,

246　2014). OTUs with sequence length <250 base pairs (over the entire amplicon length) and

247　match/sequence length <70% were excluded as potential artefacts. Each fungal genus, family

248　or order was assigned to functional categories based on FUNGuild (Nguyen et al., 2016). All

249　Glomeromycota were considered to be arbuscular mycorrhizal (AM). Taxa were considered

250　to be EcM if they matched to any sequences belonging to EcM lineages and exhibited

251　sequence blast score/sequence length above predetermined lineage-specific thresholds

252　(Tedersoo and Smith, 2017). Annotated sequence data and detailed metadata are given in

253　Supplementary Table S3 and Table S4, respectively. In order to evaluate putative host-

254　specific associations in EcM fungi, we used literature-based search for the recovered species

255　and UNITE database third-party metadata annotations at the level of SH.

256　*2.4. Statistical analysis*

257   We analyzed species richness and community composition of taxa that belonged to the

258   kingdom Fungi, focusing on EcM, saprotrophic and plant pathogenic fungi. Endophytes were

259   not analyzed separately, because they were underrepresented in the OTU matrix (<50 OTUs).

260   PERMANOVA+ (Anderson et al., 2008) was used to generate community composition

261   models for individual fungal groups and litter types (foliar and root litter). We tested the

262   effect of litter species composition, litter species richness, focal tree species identity, plot

263   (fixed factors) and block (random factor), and mass loss (covariate) on fungal community

264   composition in foliar and root litter samples. The OTU matrix was subjected to Hellinger

265   transformation using vegan package (Oksanen et al., 2017) in R prior to community analysis.

266   We compared the performance of Bray-Curtis distance and Hellinger distance (D17) metric

267   for the abundance data (including OTUs with frequency > 1) (Tedersoo et al., 2015). We

268   chose Bray-Curtis metric as models generated based on the resemblance matrices had higher

269   adjusted coefficients of determination (R^2_{adj}) values. Euclidean distance was applied to

270   generate environmental distance matrices. The best community composition model for each

271   group and litter type was constructed using PERMANOVA function in PERMANOVA+. The

272   factor block was nested in tree species and plot. Type I test and 999 permutations were used

273   for the unbalanced sampling design with nested effects. Statistical significance level was at α

274   = 0.05.

275   We used the phyloseq package (McMurdie and Holmes, 2013) in R for visualizing taxonomic

276   assignments at the order level in foliar and root litter. We used the vegan package to construct

277   Nonmetric Multidimensional Scaling (NMDS) plots for saprotrophs, plant pathogens and

278   EcM fungal group in foliar and litter samples. NMDS graphs were constructed to assess

279   differences in fungal community composition among litter species. The function metaMDS

280   with Procrustes analysis and 200 iterations was used to perform NMDS. In metaMDS, raw

281 sequence abundance matrix was square-root-transformed and subjected to Wisconsin double

282 standardization. Bray-Curtis dissimilarity was used to calculate community matrix.

283 For richness analyses of foliar and root litter biota, we calculated the standardized residuals of

284 OTU richness in relation to the square-root of the number of obtained sequences to account

285 for differences in sequencing depth (Tedersoo et al., 2014). Standardized residuals were

286 obtained from multiple regression analysis performed in STATISTICA 12 (StatSoft Inc.,

287 Tulsa, OK, USA). We used the vegan package to calculate Shannon diversity index and

288 evenness. Using the previously described nested design, mixed effects models for fungal

289 standardized residuals of OTU richness were calculated in STATISTICA. We used type I sum

290 of squares (SS) to construct the models. To test the non-additivity of decomposition rates in

291 two- and four-species mixtures, we calculated expected mass losses based on the average of

292 single species decomposition, and compared with the observed rates. We also tested if mass

293 loss was described by litter species composition, litter species richness, tree species identity,

294 plot or block. Using one-way and two-way analysis of variance (ANOVA), we performed

295 Tukey's post hoc tests to distinguish among statistically significantly different groups, using

296 the R package agricolae (De Mendiburu, 2014). To test the host-specific EcM fungal

297 preferences towards the litter of their host plant litter species, we extracted host-specific

298 fungal OTU and their sequence abundance as a relative proportion to all EcM fungi. Only

299 monospecific litter was included in testing the differences between 'home' and 'away'

300 treatments. We considered single litter species under the same tree species the 'home'

301 combination. We tested the differences in host specific EcM fungal composition in four

302 'home' combinations and 12 'away' combinations using one-way ANOVA.

303 *2.5. Accession numbers*

304   The sequence data has been submitted to the SRA database under accession number

305   SRP133556.

306

3. Results

3.1. Mass loss

Within one year of decomposition, the average mass loss of foliar and root litter were 30.4% (± 8.1%, SD) and 19.2% (± 6.8%), respectively. Litter species richness had no significant effect on mass loss of foliar litter ($F_{2,272} = 0.38$; $P = 0.682$; Fig. 1a) or root litter ($F_{2,275} = 0.51$; $P = 0.599$; Fig. 1b). Instead, litter species composition was the best predictor for decomposition rate of both foliar litter ($F_{10,264} = 6.58$; $P < 0.001$; Fig. 1c) and root litter ($F_{10,267} = 57.47$; $P < 0.001$; Fig. 1d). We did not detect any significant non-additive effects of foliar (Fig. 1c) and root litter (Fig. 1d) mixtures on decomposition rates. Unexpectedly, the slowest decomposing foliar litter combinations included deciduous litter (treatments: birch birch–spruce and alder–birch) and the fastest decomposing foliar litter treatments included coniferous litter (treatments: spruce, pine–spruce and alder–spruce) which is assumed to be more recalcitrant than deciduous litter and thus expected to decompose slower (Fig. 1c). Of root litter, coniferous litter (treatments: pine–spruce, pine and spruce) decomposed fastest, whereas deciduous litter (treatments: birch, alder–birch and alder) decomposed slowest (Fig. 1d). We detected significant differences in decomposition rates of foliar and root litter of conifers and broadleaf trees. Foliar litter of birch decomposed significantly slower than foliar litter of pine ($F_{1,95} = 37.18$; $P < 0.001$), spruce ($F_{1,95} = 52.28$; $P < 0.001$) and pine–spruce treatments ($F_{1,53} = 10.94$; $P = 0.002$). Mass loss of coniferous (treatments: pine–spruce, pine and spruce) root litter was significantly higher than that of broadleaf root litter (treatments: birch, alder–birch and alder; $F_{1,210} = 572.11$; $P < 0.001$).

There were no significant overall differences between 'home' and 'away' treatments in foliar litter ($F_{1,194} = 0.04$; $P = 0.851$) or root litter ($F_{1,196} = 0.007$; $P = 0.934$). Also, we found no significant 'home' vs 'away' effects for root litter species ($F_{3,190} = 0.73$; $P = 0.535$; Fig. 2b).

331　However, we detected a weak 'home' vs 'away' effect for foliar litter species ($F_{3,188} = 2.69$; P

332　$= 0.048$; Fig. 2a). Foliar litter of birch tended to decompose 4% faster in 'home' than 'away'

333　treatment.

334　*3.2. Identification of litter biota*

335　The 8,044,053 high-quality reads were clustered into 14,963 OTUs. Based on sequence

336　distribution in negative and positive controls, we excluded OTU sequence counts=1 per

337　sample to remove most of the potential tag-switching errors. After removal of samples with

338　<500 sequences (three samples) and OTUs with poor BLASTn values, the final data set

339　comprised of 10,042 OTUs, 7,532,808 sequences and 553 samples. In all, 29.0% of OTUs

340　occurred only in one sample. Each sample was comprised of 566 to 45,105 reads (median:

341　12,641 reads) and 45 to 1107 OTUs (median: 421 OTUs).

342　Altogether 91.1% of OTUs were assigned to Fungi, 5.3% to Viridiplantae (mostly Bryophyta,

343　Chlorophyta and Magnoliophyta), 1.4% to Metazoa (mostly Nematoda), 0.9% to Alveolata

344　(mostly Ciliophora), 0.6% to Rhizaria (mostly Cercozoa), 0.6% to Stramenopila (mostly

345　Chrysophyceae) and 0.1% to Apusozoa. Of the 9,148 fungal OTUs, 48.2% were identified as

346　Ascomycota, 20.2% as Basidiomycota, 1.3% as Chytridiomycota, 0.7% as Mucoromycota,

347　0.6% as Mortierellomycota, 0.2% as Glomeromycota, 0.2% as Rozellomycota, whereas

348　28.5% remained unclassified at phylum level. Helotiales (1,926,859 reads, 1647 OTUs),

349　Agaricales (616,174 reads, 460 OTUs), and Rhytismatales (429,495 reads, 183 OTUs) were

350　dominant orders; *Lophodermium* (404,960 reads, 127 OTUs), *Mollisia* (325,480 reads, 101

351　OTUs) and *Mycena* (226,043 reads, 107 OTUs) were dominant genera. Helotiales was

352　relatively more abundant in root litter samples, whereas Agaricales and Rhytismatales were

353　relatively more abundant in foliar litter samples, the latter especially in pine needle samples.

At fungal order level, the composition of fungal communities was not litter species composition specific (Fig. 3).

Respectively, saprotrophs, plant pathogens and EcM fungi accounted for 66.1%, 16.8% and 11.0% of OTUs that could be assigned to functional guilds. The average number of EcM OTUs was 5.5 ± 11.2 (SD) in foliar litter samples and 4.2 ± 3.6 (SD) in root litter samples. The average number of plant pathogens was 45.1 ± 23.2 (SD) in foliar litter samples and 17.3 ± 10.9 (SD) in root litter samples. The average number of saprotrophs was 121.9 ± 64.5 (SD) in foliar litter samples and 84.7 ± 37.2 (SD) in root litter. In foliar and root litter samples, altogether 344 and 163 EcM fungal OTUs were recognized. The EcM fungal community included an estimated 9.7% of host-specific OTUs that corresponded to 33.8% of total EcM fungal sequences. Among these, alder-specific fungi comprised 16 OTUs (42% of host-specific EcM fungal OTUs) with 20,569 sequences (87% of host-specific fungal sequences).

3.3. Fungal community structure

Community structure of all fungi, saprotrophic and plant pathogenic fungi was best described by litter species composition and litter species richness in foliar litter (Table 1). Litter species composition had the strongest effect on the community structure of saprotrophs ($F_{pseudo} = 10.87$; $R^2_{adj} = 0.210$; $P < 0.001$; Fig. 4a) and plant pathogens ($F_{pseudo} = 16.64$; $R^2_{adj} = 0.292$; $P < 0.001$; Fig. 4c), but negligible effect on EcM fungi in foliar litter ($F_{pseudo} = 1.47$; $R^2_{adj} = 0.003$; $P < 0.001$; Fig. 4e). In root litter, no clear influence of litter species composition was evident for fungal composition (Table 1). Community composition of all fungi in root litter was marginally influenced by litter species richness ($F_{pseudo} = 3.18$; $R^2_{adj} = 0.011$; $P < 0.001$). Saprotrophs were weakly affected by litter species composition ($F_{pseudo} = 2.92$; $R^2_{adj} = 0.048$; $P < 0.001$; Fig. 4b), whereas plant pathogens were slightly affected by plot ($F_{pseudo} = 4.51$; $R^2_{adj} = 0.017$; $P < 0.001$; Fig. 4d) and EcM fungi were affected by focal tree species identity

378   ($F_{\text{pseudo}} = 2.50$; $R^2_{\text{adj}} = 0.025$; $P < 0.001$; Fig. 4f). Focal tree species identity and interaction

379   term between tree and litter species composition had a significant but weak effect on

380   composition of most fungal guilds both in foliar and root litter (Table 1).

3.4. Fungal richness

382   The mean OTU richness of all fungi in foliar and root litter samples was 426.3 ± 167.0 (SD)

383   and 345.1 ± 145.7 (SD), respectively. Mixed effects models for standardized residuals of

384   OTU richness indicated that the best predictors differed among functional groups of fungi

385   depending on litter type (Table 2). Litter species richness was one of the two strongest

386   predictors for total fungal (positive effect; $F_{2,195} = 83.38$; $R^2_{\text{adj}} = 0.229$; $P < 0.001$; Fig. 5a),

387   saprotroph (positive effect; $F_{2,195} = 22.27$; $R^2_{\text{adj}} = 0.099$; $P < 0.001$) and plant pathogen

388   richness ($F_{2,195} = 87.99$; $R^2_{\text{adj}} = 0.251$; $P < 0.001$) in foliar litter, but none of the tested factors

389   affected richness of EcM fungi. In foliar litter, litter species composition had a significant

390   additional effect on richness of all fungi (positive effect; $F_{8,195} = 16.82$; $R^2_{\text{adj}} = 0.209$; $P <$

391   0.001), plant pathogens (positive effect; $F_{8,195} = 24.68$; $R^2_{\text{adj}} = 0.284$; $P < 0.001$) and

392   saprotrophs (positive effect; $F_{8,195} = 6.96$; $R^2_{\text{adj}} = 0.118$; $P < 0.001$). Shannon diversity index

393   of all fungi was significantly affected by litter species composition ($F_{10,264} = 15.31$; $P < 0.001$)

394   and litter species richness ($F_{2,272} = 14.62$; $P < 0.001$) in foliar litter. The Shannon diversity

395   index showed that fungal diversity increased with increasing number of litter species in a

396   mixture. In foliar litter, the evenness values were similar at the three levels of litter richness

397   ($F_{2,272} = 0.60$; $P = 0.549$), but differed by litter species composition ($F_{10,264} = 8.12$; $P < 0.001$).

398   We detected significant non-additive effects of foliar litter mixtures on fungal richness (Fig.

399   5c). Three out of the seven foliar litter mixtures showed significant synergistic effects: two-

400   species mixtures alder–birch ($t = 2.11$; $P = 0.037$) and birch–spruce ($t = 2.61$; $P = 0.011$) and

401   the four-species mixture alder–birch–pine–spruce ($t = 9.36$; $P < 0.001$).

402   Richness of root litter had weaker effects on fungal OTU richness than richness of foliar litter

403   (Fig. 5b). Richness of root litter increased OTU richness of saprotrophs ($F_{2,233} = 11.15$; $R^2_{adj} =$

404   0.058; $P < 0.001$), plant pathogens ($F_{2,233} = 7.71$; $R^2_{adj} = 0.039$; $P < 0.001$) and EcM fungi

405   ($F_{2,198} = 5.42$; $R^2_{adj} = 0.024$; $P = 0.005$). Litter species composition had a significant positive

406   effect on richness of EcM fungi ($F_{8,198} = 2.25$; $R^2_{adj} = 0.023$; $P = 0.026$), plant pathogens

407   ($F_{8,233} = 2.62$; $R^2_{adj} = 0.035$; $P = 0.009$) and saprotrophs ($F_{8,233} = 2.15$; $R^2_{adj} = 0.022$; $P =$

408   0.032) in root litter. In root litter, the Shannon diversity index of all fungi was significantly

409   affected by litter species composition ($F_{10,267} = 2.89$; $P = 0.002$), but remained unaffected by

410   litter species richness ($F_{2,275} = 1.55$; $P = 0.213$). The evenness was influenced by litter species

411   richness ($F_{2,275} = 6.50$; $P = 0.002$) and litter species composition ($F_{10,267} = 6.22$; $P < 0.001$) in

412   root litter. We detected significant non-additive effects of root litter mixtures on fungal

413   richness (Fig. 5d). Two out of the seven root litter mixtures showed significant synergistic

414   effects: the two-species mixtures alder–birch ($t = 2.24$; $P = 0.028$) and birch–spruce ($t = 2.37$;

415    $P = 0.020$).

416   *3.5. EcM specificity analysis*

417   We assigned OTUs of EcM fungi into host-specific and promiscuous taxa to test preferences

418   of fungi for the litter of their intimate hosts. Relative sequence abundance and OTU

419   abundance of host-specific EcM fungi in 'home' combinations were similar to 'away'

420   combinations in both foliar ($F_{1,145} = 0.00004$; $P = 0.995$ and $F_{1,145} = 0.42$; $P = 0.518$,

421   respectively) and root litter ($F_{1,118} = 0.86$; $P = 0.355$ and $F_{1,118} = 1.34$; $P = 0.249$,

422   respectively). Taken separately, the four 'home' and 12 'away' combinations had no

423   significant effect on relative sequence abundance of host-specific EcM fungi in either foliar

424   litter ($F_{15,131} = 1.56$; $R^2 = 0.12$; $P = 0.093$; Supplementary Fig. S1a) or root litter ($F_{15,104} =$

425   0.63; $R^2 = 0.07$; $P = 0.842$; Supplementary Fig. S1b). By contrast, these combinations had a

426   weak but significant effect on relative host-specific EcM fungal OTU abundance in foliar

20

427   litter ($F_{15,131}$ = 2.50; R^2 = 0.14; P = 0.003; Supplementary Fig. S1c) but not in root litter

428   ($F_{15,104}$ = 1.23; R^2 = 0.11; P = 0.264; Supplementary Fig. S1d). Alder-specific fungal taxa

429   contributed most to the relative OTU and sequence abundance both in foliar and in root litter,

430   but these taxa showed no evidence for preference of alder litter.

431

4. Discussion

4.1. Litter mass loss

Decomposition rates of both foliar and root litter remained unaffected by litter species richness, providing no support to the first hypothesis. Non-additive patterns of mass loss are common (Gartner and Cardon, 2004; Lecerf et al., 2011; Cuchietti et al., 2014; Santonja et al., 2015), by contrast this finding is in agreement with studies that have reported additive decomposition rates (Prescott et al., 2000; Hoorens et al., 2010; Guerrero-Ramírez et al., 2016), suggesting that mass loss dynamics of monospecific treatments can be used to predict the dynamics of mixed species treatments. Mass loss of foliar and root litter was most strongly affected by litter species composition, supporting the view that litter quality may play a strong role in decomposition (Hoorens et al., 2010; Coq et al., 2011; Cuchietti et al., 2014; Handa et al., 2014; Setiawan et al., 2016; Dawud et al., 2017). Broadleaf litter usually decomposes faster than coniferous litter (Prescott et al., 2000; Silver and Miya, 2001), but this may strongly depend on studied species (Cornelissen et al., 2001; Hobbie et al., 2010, 2006). In line with Heim and Frey (2004), we found that decomposition of coniferous root litter was more rapid than decomposition of broadleaf root litter. This may indicate some home-field advantage in these plantations as much of the litter on the forest floor is dominated by needle litter of the three conifers from previous years (personal observations). However, we detected no clear home-field advantage effects, with no support to the second hypothesis. Although tree effects are found to be largest close to the trunk (Saetre and Bååth, 2000), all five tree species, growing together for 15 years, may have had effects on soil properties and microbial community and may not have allowed 'away' to distinguish from 'home' in this study system.

4.2. Fungal richness and composition

We found that saprotrophs and putative plant pathogens dominated both foliar and root litter, which is consistent with previous findings in Pinaceae-dominated forests (Herzog, 2017; Kyaschenko et al., 2017). Within one year of decomposition, many of the typical leaf pathogenic fungi were evident, indicating that part of the decomposer community most probably originated from the living tissue. Plant pathogens have strong preferences for litter species, with particularly strong patterns between coniferous and broadleaf foliage (Zhou and Hyde, 2001; Arnold, 2007; Prescott and Grayston, 2013).

Consistent with the third hypothesis, foliar litter species composition affected the composition of associated fungal communities. We detected that the effect was stronger on communities of saprotrophic and plant pathogenic fungi compared with EcM fungi. Plant pathogens and especially saprotrophs exhibit higher specialization for acquisition of nutrients from recalcitrant plant-derived biopolymers (Zhou and Hyde, 2001; Baldrian, 2016). In root litter, species composition effect was much weaker, which could be related to our heating treatment, more similar root chemistry or lower specificity of soil-borne root decomposers. In root litter, broadleaved trees differed from conifers in their fungal composition that may be explained by different chemistry or its confounding phylogeny effect (Betulaceae *vs*. Pinaceae).

Litter species richness and litter species composition were two major predictors for explaining species richness of saprotrophs and plant pathogens in foliar litter. In foliar litter, we detected non-additive effects of two and four litter species mixtures on fungal richness, whereas in root litter, litter species mixtures had non-additive effect on fungal diversity only in two-species mixture, which is only partly consistent with our fourth hypothesis. Several authors have reported enhanced fungal diversity with increasing foliar litter diversity (Kubartová et al., 2009; Chapman and Newman, 2010; Santonja et al., 2017). This can be explained by higher niche and resource availability or fine-scale resource heterogeneity (Chapman and Newman, 2010). The lack of positive root litter richness effect can be partly explained by the initial

480   heating treatment for endophytes and pathogens, or the paucity of specialist fungi for fine root

481   decomposition, because roots naturally decompose in the soil matrix (Štursová et al., 2012).

482   Our fifth hypothesis predicted that host-specific EcM fungi are more litter species-specific

483   than generalists, preferring the litter of their host plant. Based on high host-specificity and

484   restricted EcM fungal community of alders (Tedersoo et al., 2009; Põlme et al., 2013), we

485   expected that alder-specific fungi, in particular, occur in relatively greater abundance in alder

486   litter. Although the preference of EcM fungi to certain litter species has been previously

487   demonstrated (Conn and Dighton, 2000), we found no support to this hypothesis. No

488   differences in litter species preference in EcM fungi in general or preference for intimate

489   host's litter in particular was detected. However, it cannot be excluded that fresh litter is

490   unsuitable substrate for EcM fungi or the scale of our study is too small.

491

5. Conclusions

Our results showed that litter species composition was the main driver of decomposition rate and saprotroph and pathogen community composition in foliar litter. However, EcM fungal communities both in foliar and root litter were only marginally affected by litter species composition. Also, we found no evidence for the positive feedback of host litter on performance of EcM fungi specific to particular hosts. These results collectively suggest that growth of EcM fungal hyphae into fresh litter is rather opportunistic and unspecific. Litter species composition and litter species richness were two major factors underlying fungal richness in foliar litter. In particular, we detected strong synergistic effect of four-species foliar litter mixture on fungal richness. This study demonstrates that the fungal community in both foliar and root litter is affected by litter species richness and composition.

Author contributions

L.T., J.K., S.B and E.O. designed the experiment; E.O. conducted fieldwork and molecular analysis; S.A. performed bioinformatic analysis; E.O. annotated metadata, analyzed the data, and wrote the first draft of the manuscript; all authors contributed to writing of the manuscript.

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853

854 **Fig. 1.** Litter mass loss (%) of (a, c) foliar and (b, d) root litter described by (a, b) litter species

855 richness (1, monospecific; 2, two-species mixtures; 4, four-species mixture), and (c, d)

856 observed and expected decomposition rates (%) for litter species composition (A, alder; B,

857 birch; P, pine; S, spruce; AB, alder–birch; AP, alder–pine; AS, alder–spruce; BP, birch–pine;

858 BS, birch–spruce; PS, pine–spruce; ABPS, alder–birch–pine–spruce). Data represents the

859 means of all samples with standard errors. Different letters denote significant differences

860 among factor levels. Expected litter mass loss rates are displayed with white and observed

861 with black.

862 **Fig. 2.** 'Home' versus 'away' treatment effect on mass loss (%) in (a) foliar and (b) root litter

863 described by litter species identity. Data represents the means of all samples with standard

864 errors. Different letters denote significant differences among factor levels. 'Away' treatments

865 are displayed with white and 'home' treatments with black.

866 **Fig. 3.** Abundances of major fungal orders in (a) foliar and (b) root litter. The data represents

867 the mean values of the relative abundances of ITS2 amplicons expressed as percentages. A,

868 alder; B, birch; P, pine; S, spruce; AB, alder–birch; AP, alder–pine; AS, alder–spruce; BP,

869 birch–pine; BS, birch–spruce; PS, pine–spruce; ABPS, alder–birch–pine–spruce.

870 **Fig. 4.** NMDS ordination plots describing the relative importance of litter species composition

871 in explaining the community structure of (a, b) saprotrophs, (c, d) plant pathogens and (e, f)

872 EcM fungi in (a, c, e) foliar and (b, d, f) root litter. Ellipses denote 95% confidence intervals

873 around the mean values of litter species composition. Circles, monospecific litters; stars,

874 alder–birch mixture; diamonds, alder–pine mixture; upward triangles, alder–spruce mixture;

875 crosses, birch–pine mixture; pluses, birch–spruce mixture; downward triangles, pine–spruce

876 mixture; red squares, alder–birch–pine–spruce mixture. Blue, alder; green, birch; pink, pine;

877 yellow, spruce.

878   **Fig. 5.** Variation in standardized residual richness of all fungi in (a, c) foliar and (b, d) root

879   litter described by (a, b) litter species richness (1, monospecific; 2, two-species mixtures; 4,

880   four-species mixture) and (c, d) litter species composition (A, alder; B, birch; P, pine; S,

881   spruce; AB, alder–birch; AP, alder–pine; AS, alder–spruce; BP, birch–pine; BS, birch–spruce;

882   PS, pine–spruce; ABPS, alder–birch–pine–spruce). Data represents the means of all samples

883   with standard errors. Different letters denote significant differences among factor levels.

884   Expected standardized residual richness of all fungi is displayed with white and observed with

885   black.

886 **Table 1.** Multivariate models for community composition of all fungi, EcM, plant pathogen

887 and saprotroph group in foliar and root litter

Foliar litter				Root litter			
variable	R^2 adj	Pseudo-F	P-value	variable	R^2 adj	Pseudo-F	P-value
1 Fungi (total)							
Litter composition	0.209	11.00	<0.001	Litter richness	0.011	3.18	<0.001
Litter richness	0.020	5.45	<0.001	Litter composition	0.050	3.06	<0.001
Plot	0.006	2.21	<0.001	Plot	0.013	2.98	<0.001
Tree	0.014	2.04	<0.001	Tree	0.032	2.64	<0.001
Block (Tree*Plot)	0.016	1.66	<0.001	Block (Tree*Plot)	0.063	1.85	<0.001
Mass loss	0.000	1.31	0.010	Mass loss	0.001	1.27	0.015
Tree*Litter composition	-0.026	1.17	<0.001	Tree*Litter composition	-0.019	1.05	0.015
1.1 EcM fungi							
Plot	0.027	3.37	<0.001	Tree	0.025	2.50	<0.001
Block (Tree*Plot)	0.184	3.12	<0.001	Plot	0.004	1.64	0.027
Litter richness	0.001	1.58	<0.001	Block (Tree*Plot)	0.023	1.20	<0.001
Tree	0.029	1.47	<0.001	Litter composition	-0.004	0.93	0.803
Litter composition	0.003	1.47	<0.001	Litter richness	-0.003	0.71	0.975
Tree*Litter composition	-0.030	1.05	0.161	Tree*Litter composition	-0.017	0.92	0.965
1.2 Plant pathogens							
Litter composition	0.292	16.64	<0.001	Plot	0.017	4.51	<0.001
Litter richness	0.024	6.92	<0.001	Litter composition	0.050	3.01	<0.001
Tree	0.006	1.76	<0.001	Tree	0.025	2.72	<0.001
Block (Tree*Plot)	-0.018	1.40	<0.001	Litter richness	0.008	2.52	<0.001
Plot	0.001	1.38	0.049	Block (Tree*Plot)	0.028	1.44	<0.001
Tree*Litter composition	-0.025	1.28	<0.001	Tree*Litter composition	-0.012	1.07	0.043
1.3 Saprotrophs							
Litter composition	0.210	10.87	<0.001	Litter composition	0.048	2.92	<0.001
Litter richness	0.019	5.19	<0.001	Plot	0.009	2.49	<0.001
Tree	0.010	1.78	<0.001	Litter richness	0.007	2.34	<0.001
Plot	0.003	1.76	0.002	Tree	0.023	2.23	<0.001
Block (Tree*Plot)	0.011	1.57	<0.001	Block (Tree*Plot)	0.057	1.73	<0.001
Mass loss	0.000	1.37	0.004	Mass loss	0.001	1.36	0.008
Tree*Litter composition	-0.027	1.12	<0.001	Tree*Litter composition	-0.018	1.02	0.247

888

889

890   **Table 2.** The best predictors for standardized residuals of OTU richness of all fungi, EcM,

891   plant pathogen and saprotroph group

Foliar litter				Root litter			
variable	R^2 adj	*F*-value	*P*-value	variable	R^2 adj	*F*-value	*P*-value
1 Fungi (total)							
Litter richness	0.229	83.38	<0.001	Plot	0.072	15.94	<0.001
Litter composition	0.209	16.82	<0.001	Litter richness	0.005	2.09	0.127
Plot	0.017	4.51	0.040	Block (Tree*Plot)	0.050	1.61	0.020
Block (Tree*Plot)	0.037	3.19	<0.001	Tree*Litter composition	0.015	1.38	0.103
Tree*Litter composition	-0.046	1.63	0.027	Litter composition	-0.000	1.22	0.288
1.1 EcM fungi							
Litter composition	-0.013	0.50	0.855	Litter richness	0.024	5.42	0.005
Litter richness	-0.007	0.09	0.913	Block (Tree*Plot)	0.148	2.43	<0.001
Tree*Litter composition	-0.094	0.28	1.000	Litter composition	0.023	2.25	0.026
				Tree*Litter composition	-0.020	1.06	0.385
1.2 Plant pathogens							
Litter richness	0.251	87.99	<0.001	Mass loss	0.037	13.44	<0.001
Litter composition	0.284	24.68	<0.001	Litter richness	0.039	7.71	<0.001
Tree	0.014	3.60	0.022	Litter composition	0.035	2.62	0.009
Block (Tree*Plot)	-0.056	1.58	0.028	Tree*Litter composition	0.046	1.51	0.045
Tree*Litter composition	-0.069	1.11	0.330				
1.3 Saprotrophs							
Litter richness	0.099	22.27	<0.001	Litter richness	0.058	11.15	<0.001
Litter composition	0.118	6.96	<0.001	Mass loss	0.027	10.47	0.001
Block (Tree*Plot)	0.038	1.91	0.002	Litter composition	0.022	2.15	0.032
Tree*Litter composition	0.008	1.64	0.025	Tree*Litter composition	0.060	1.78	0.008

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894 **Supplementary Information**

895 **Fig. S1.** Host-specific EcM fungal preferences towards foliar and root litter species.

896 **Table S1.** Initial chemical composition of foliar and root litter species

897 **Table S2.** Primers used in this study

898 **Table S3.** OTU table of all taxa associated with foliar and root litter

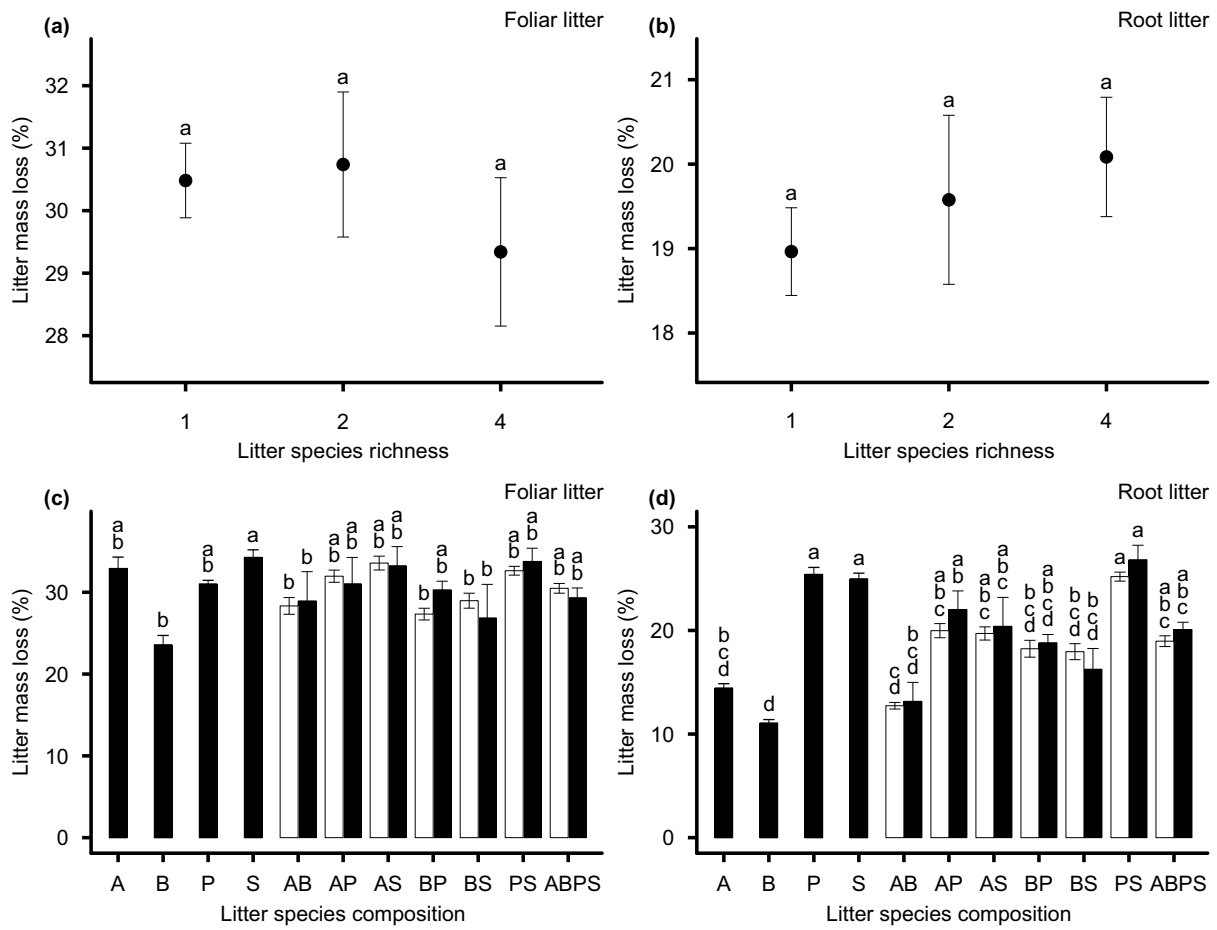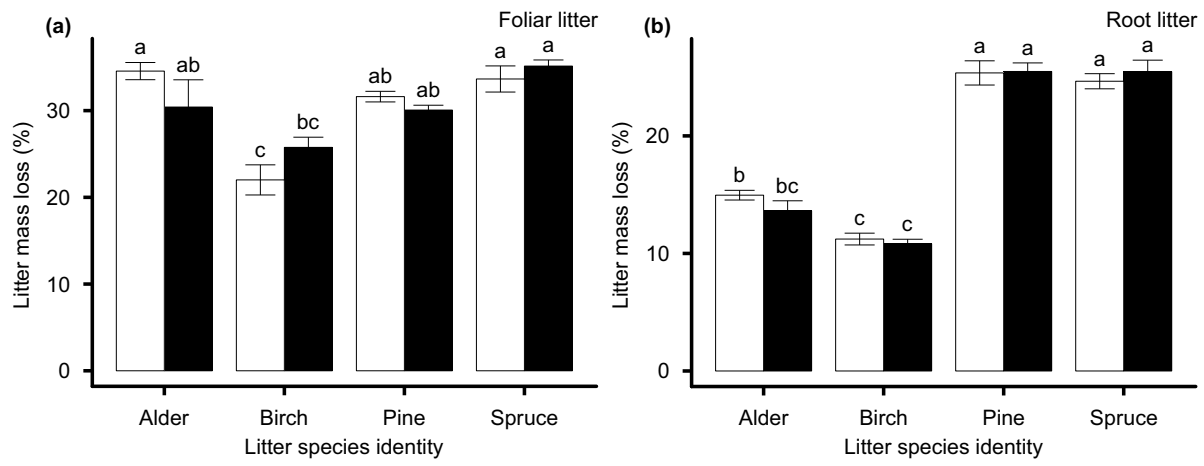899 **Table S4.** Detailed metadata for foliar and root litter samples

Figure 1

Figure 2

(a) Foliar litter — Relative sequence abundance (%) vs Litter species composition (A B P S AB AP AS BP BS PS ABPS)

(b) Root litter — Relative sequence abundance (%) vs Litter species composition (A B P S AB AP AS BP BS PS ABPS)

Legend (a): Unknown Fungi, Other Fungi, Other Ascomycota, Other Basidiomycota, Agaricales, Atheliales, Auriculariales, Boletales, Cantharellales, Capnodiales, Chaetothyriales, Dothideales, Filobasidiales, Geastrales, Helotiales, Hypocreales, Pezizales, Pleosporales, Rhytismatales, Russulales, Saccharomycetales, Sebacinales, Sporidiobolales, Thelephorales, Trechisporales, Tremellales, Venturiales, Xylariales

Legend (b): Unknown Fungi, Other Fungi, Other Ascomycota, Other Basidiomycota, Agaricales, Atheliales, Auriculariales, Boletales, Cantharellales, Coniochaetales, Corticiales, Cystofilobasidiales, Dothideales, Filobasidiales, Geastrales, Helotiales, Hypocreales, Leucosporidiales, Mucorales, Pezizales, Pleosporales, Polyporales, Rhytismatales, Russulales, Sebacinales, Thelephorales, Trechisporales, Xylariales

Figure 4

Figure 5